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**AD-P008 765**



**ACUTE AND CHRONIC RESPIRATORY LESIONS INDUCED BY SULFUR  
MUSTARD IN GUINEA PIGS : ROLE OF TACHYKININS**

**J.H. CALVET, G. TROUILLER AND A. HARF\*.**

Service de Pharmacologie Centre d'Etudes du Bouchet  
91710, Vert-le Petit, France.

\*Département de Physiologie, INSERM U296, Faculté de Médecine de Créteil  
94010, Créteil, France

We investigated in anesthetized guinea pigs the involvement of tachykinins in respiratory alterations after an airway intoxication by sulfur mustard (SM). Early lesions were evaluated after 5h. Respiratory system resistance (R) and compliance were measured by the occlusion method and airway microvascular permeability by measuring the Evans Blue dye concentration in the trachea and main bronchi. Two groups of animals were studied treated with capsaicin (which induces a tachykinin depletion) or by its vehicle. Capsaicin pretreatment had no effect on the measured parameters.

We also measured 14 J after the intoxication tracheal epithelium neutral endopeptidase (NEP) (the main enzyme which degrades tachykinins). In addition bronchial responsiveness to exogenous substance P was studied in two groups of animals intoxicated with SM or not. Tracheal epithelium NEP activity was decreased from  $0.448 \pm 0.027$  nmol.min<sup>-1</sup>.mg protein<sup>-1</sup> in controls to  $0.182 \pm 0.038$  in intoxicated animals. Response to substance P was greater in intoxicated animals with  $R=2.98 \pm 1.57$  cmH<sub>2</sub>O.ml<sup>-1</sup>.s versus  $0.35 \pm 0.02$  in controls, after  $5.10^{-5}$  M aerosolized substance P.

These results suggest tachykinins are not preponderant in the early stage lesions but that bronchial hyperactivity is present at recovery, related to epithelium NEP depletion.

**94-07927**



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## INTRODUCTION

Sulfur mustard (SM ; bis-2 chloroethyl sulfide) was initially used as a vesicant chemical warfare agent during World War 1. Its use in the past decade and the growing capacity for the manufacture of chemical weapons have increased the risk that soldiers or civilians may be exposed to SM. At the early stage after inhalation, this alkylating agent has been reported to cause mild to severe laryngeal and tracheo-bronchial inflammation, with possible necrosis of the epithelium (7). The delayed toxic effects of SM on the respiratory tract of severely injured people include asthma-like symptoms which have been shown to persist for at least two years after the initial exposure to SM (8).

We carried out this study in guinea pigs to investigate the functional alterations in the airways induced by intratracheal injection of SM. Firstly, we explored the acute respiratory effects of the resulting intoxication. Because the functional alterations we observed suggested neurogenic inflammation, we evaluated the role of sensory nerves in the acute effects of SM by studying the effect of treatment with capsaicin before SM injection. Next, we assessed the effects of SM-intoxication on airway responsiveness 14 days after exposure. Because we found airway hyperresponsiveness to substance P, we explored the possible association of this hyperresponsiveness with a decrease in the activity of neutral endopeptidase (NEP)-in the tracheal epithelium, as NEP is known to play an important role in modulating the effects of substance P in the airways (1).

## METHODS

Experiments were performed on male Hartley strain guinea pigs weighing 250-300g (Charles River, France), which were housed in air-filtered temperature-controlled units (21°C) with food and water freely available.

### SM administration

SM was administered intratracheally as follows: guinea pigs were briefly anesthetized with fluothane via a face mask. Cervicotomy was performed and a drop of 1% lidocaine was instilled subcutaneously (s.c.). The trachea was gently exposed and 0.3 ml/kg of either SM or ethanol (SM solvent) was injected into the trachea through a No. 24g needle. Cervicotomy was closed by two agraffes. Within 10 min of anesthesia, all guinea pigs had recovered. For security reasons, injections were performed in a controlled area under an adequate hood, with a mask and buthyl gloves, in accordance with the security regulations of our institution.

### Experimental protocol

#### *Early stage studies (5 h after intoxication)*

Respiratory system resistance and compliance, blood gases and airway microvascular permeability were measured in 5 guinea pigs 5 h after intratracheal injection of SM and in 5 controls injected with ethanol. To establish whether capsaicin-sensitive nerves were involved in the acute respiratory effects of SM, we treated 10 other guinea pigs with a single s.c. injection of 50 mg/kg capsaicin, 10 days before SM intoxication, using the method described by Lundberg *et al.* (16). Ten days later, these guinea pigs were randomly allocated to intratracheal injection of SM or ethanol.

***Recovery period studies (14 days after intoxication)***

Airway responsiveness was studied 14 days after SM intoxication. We first investigated the airway responsiveness to aerosolized substance P with and without pretreatment with the NEP inhibitor, phosphoramidon. For this purpose, two groups of 12 guinea pigs each were respectively injected intratracheally with SM and ethanol. Fourteen days later, 6 of the guinea pigs in each group were randomly allocated to intratracheal injection of either 0.1ml  $10^{-4}$ M phosphoramidon or 0.9% NaCl. Twenty min after injection, airway responsiveness to substance P ( $10^{-5}$ M to  $5 \cdot 10^{-5}$ M) was measured as described above.

In addition, NEP activity in the tracheal epithelium was measured in two groups of 6 guinea pigs each, respectively injected intratracheally with SM and ethanol.

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## RESULTS

### Early stage studies (5 h after intoxication)

#### *Respiratory effects of SM-intoxication*

In intoxicated guinea pigs, SM induced a three-fold increase in respiratory system resistance, accompanied by a significant decrease in compliance. Arterial  $PO_2$  dropped significantly and  $PCO_2$  rose. SM-intoxication also caused a two-fold increase in airway microvascular permeability (Table 1).

Pretreatment with capsaicin did not prevent the acute respiratory effects of SM-intoxication (Table 1).

### Recovery period studies (14 days after intoxication)

#### *Substance P challenge*

Before the concentration-response curve for substance P was recorded, respiratory system resistance did not differ significantly in the four groups of guinea pigs tested. In the absence of pretreatment with phosphoramidon, aerosolized substance P caused weak and nonsignificant bronchoconstriction in control guinea pigs injected with ethanol. In contrast, substance P induced concentration-dependent bronchoconstriction in guinea pigs injected with SM ( $P < 0.01$ , Fig. 1). After pretreatment with phosphoramidon, aerosolized substance P induced concentration-dependent bronchoconstriction, both in guinea pigs injected with ethanol and those injected with SM. However, this bronchoconstriction was significantly greater in those injected with SM ( $P < 0.05$ ). Pretreatment with phosphoramidon significantly increased airway responsiveness to substance P in guinea pigs injected with SM ( $P < 0.05$ , Fig. 1).

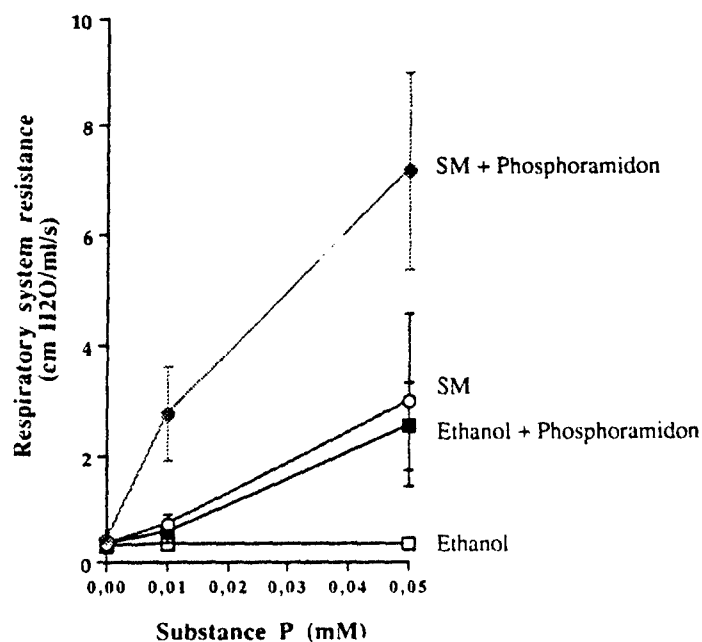
#### *NEP activity in the tracheal epithelium.*

NEP activity in the tracheal epithelium decreased significantly after SM administration (Fig. 2).

**Table 1 :** Respiratory system resistance and compliance,  $PO_2$ ,  $PCO_2$  and Evans blue dye concentration measured in guinea pigs 5 h after intratracheal injection of sulfur mustard or ethanol. Some of the guinea pigs were pretreated with capsaicin.

	No pretreatment		Pretreatment with capsaicin	
	Ethanol (n = 5)	Sulfur mustard (n = 5)	Ethanol (n = 5)	Sulfur mustard (n = 5)
Resistance ( $cmH_2O/(ml/s)$ )	$0.31 \pm 0.01$	$1.03 \pm 0.11^*$	$0.32 \pm 0.03$	$0.93 \pm 0.22^*$
Compliance ( $(ml/cmH_2O)/kg$ )	$1.04 \pm 0.04$	$0.74 \pm 0.05^*$	$0.99 \pm 0.06$	$0.72 \pm 0.06^*$
$PO_2$ (mmHg)	$88 \pm 3$	$61 \pm 6^*$	$90 \pm 10$	$57 \pm 6^*$
$PCO_2$ (mmHg)	$40 \pm 2$	$44 \pm 2^*$	$38 \pm 3$	$52 \pm 3^*$
Evans blue dye extravasation (ng/mg wet weight tissue)	$36 \pm 4$	$72 \pm 10^*$	$38 \pm 14$	$73 \pm 13^*$

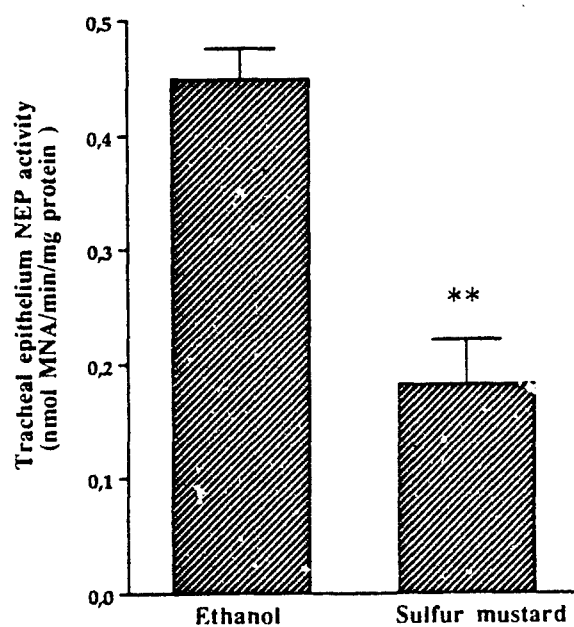
Results are means  $\pm$  SEM. \*  $P < 0.05$  between guinea pigs injected with SM and those injected with ethanol



**Figure 1:** Concentration-response curves recorded after exposure to aerosolized substance P of guinea pigs injected intratracheally with either SM or ethanol 14 days before recording. Some of them were then treated with phosphoramidon. In the absence of pretreatment with phosphoramidon, bronchoconstriction in response to substance P was greater in guinea pigs injected with SM than in those injected with ethanol ( $P < 0.05$ ). After pretreatment with phosphoramidon, bronchoconstriction in response to substance P was also greater in SM-intoxicated guinea pigs than in those injected with ethanol ( $P < 0.05$ ). Pretreatment with phosphoramidon significantly increased airway responsiveness to substance P in guinea pigs injected with SM ( $P < 0.05$ ).



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**Figure 2:** NEP activity in the tracheal epithelium of guinea pigs injected intratracheally with either SM or ethanol 14 days before measurement. \*\*P < 0.002.

## DISCUSSION

Our results demonstrate that in the guinea pig, SM induces marked bronchoconstriction 5 h after intratracheal administration. Fourteen days thereafter, the airways are hyperresponsive to substance P.

As regards the marked acute bronchoconstriction, the decrease in arterial  $PO_2$  and the increase in tracheobronchial microvascular permeability observed here 5 hours after exposure to SM, they were not prevented by pretreatment with capsaicin, suggesting that capsaicin-sensitive nerves are either not involved, or are not the main factor, in the functional alterations observed at this early stage. It was previously reported that sensitive denervation did not prevent the skin lesions induced by sulfur mustard (4). The local production of inflammatory mediators induced by epithelial cell destruction, as reported in skin lesions (5), might explain the functional alterations we observed here after intratracheal injection of SM.

In man, the follow-up of a series of severely injured SM victims indicated that 78% experienced persistent respiratory effects, mainly asthma-like symptoms (8). Our study shows that in guinea pigs, SM-intoxication induces airway hyperresponsiveness to exogenous substance P 14 days after SM exposure. This hyperresponsiveness to substance P has also been observed after other types of intoxication, like that caused by tobacco smoke, toluene-diisocyanate or ozone, and was attributed to a decrease in the tracheal activity of NEP (2, 6, 3), the main enzyme which degrades tachykinins in the airways (1). In our study, we found that tracheal NEP activity decreased after SM-intoxication. NEP is a membrane-bound enzyme located in the basal and ciliated tracheal cells, as shown by immunologic methods. It is worth noting that, unlike previous studies which demonstrated a decrease in NEP activity in the tracheal epithelium immediately after exposure to irritants, our study is the first to demonstrate that this decrease persists fourteen days after intoxication.

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